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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US96/18788 <b>(22) International Filing Date:</b> 21 November 1996 (21.11.96)  <b>(30) Priority Data:</b> 08/564,491 29 November 1995 (29.11.95) US  <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> US 08/564,491 (CON) Filed on 29 November 1995 (29.11.95)  <b>(71) Applicant (for all designated States except US):</b> HANDELMAN, Joseph, H. [US/US]; 26 West 61st Street, New York, NY 10023 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SHANDER, Douglas [US/US]; 16112 Howard Landing Drive, Gaithersburg, MD 20878 (US). FUNKHOUSER, Margaret [US/US]; 1307 Hickory Creek Court, Great Falls, VA 22066 (US). HENRY, James [US/US]; 6776 Wood Duck Court, Frederick, MD 21701 (US). AHLUWALIA, Gurpreet [US/US]; 8632 Stabview Court, Gaithersburg, MD 20852 (US).		<b>(74) Agents:</b> HANDELMAN, Joseph, H.; Ladas & Parry, 26 West 61st Street, New York, NY 10023 (US) et al.  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> REDUCTION OF HAIR GROWTH  <b>(57) Abstract</b>  Mammalian hair growth is reduced by applying to the skin a dermatologically acceptable composition including an inhibitor of arginase.		

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### REDUCTION OF HAIR GROWTH

The invention relates to a method of reducing unwanted hair growth in mammals.

A main function of mammalian hair is to  
5 provide environmental protection. However, that  
function has largely been lost in humans, in whom hair  
is kept or removed from various parts of the body  
essentially for cosmetic reasons. For example, it is  
generally preferred to have hair on the scalp but not  
10 on the face.

Various procedures have been employed to  
remove unwanted hair, including shaving, electrolysis,  
depilatory creams or lotions, waxing, plucking, and  
therapeutic antiandrogens. These conventional  
15 procedures generally have drawbacks associated with  
them. Shaving, for instance, can cause nicks and cuts,  
and can leave a perception of an increase in the rate  
of hair regrowth. Shaving also can leave an  
undesirable stubble. Electrolysis, on the other hand,  
20 can keep a treated area free of hair for prolonged  
periods of time, but can be expensive, painful, and  
sometimes leaves scarring. Depilatory creams, though  
very effective, typically are not recommended for  
frequent use due to their high irritancy potential.  
25 Waxing and plucking can cause pain, discomfort, and  
poor removal of short hair. Finally, antiandrogens  
-- which have been used to treat female hirsutism --  
can have unwanted side effects.

It has previously been disclosed that the

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rate and character of hair growth can be altered by applying to the skin inhibitors of certain enzymes. These inhibitors include inhibitors of 5-alpha reductase, ornithine decarboxylase, S-adenosylmethionine decarboxylase, gamma-glutamyl transpeptidase, and transglutaminase. See, for example, Breuer et al., U.S. Pat. No. 4,885,289; Shander, U.S. Pat. No. 4,720,489; Ahluwalia, U.S. Pat. No. 5,095,007; Ahluwalia et al., U.S. Pat. No. 5,096,911; Shander et al., U.S. Pat. No. 5,132,293; and Shander et al., U.S. Pat. No. 5,143,925.

Arginase (S-argine aminohydrolase EC 3.5.3.1) catalyzes the hydrolysis of L-arginine into ornithine and urea.

It has now been found that unwanted mammalian (including human) hair growth -- particularly androgenstimulated hair growth -- can be reduced by applying to the skin a dermatologically acceptable composition including an inhibitor of arginase in an amount effective to reduce hair growth. The unwanted hair growth which is reduced may be normal hair growth, or hair growth that results from an abnormal or diseased condition.

Preferred inhibitors of arginase include alpha aminoisobutyric acid (Bedford et al., 1988 Proceedings of the Society for Experimental Biology and Medicine 188, 509-14); N-G-hydroxy-L-arginine (Daghigh et al., 1994 Biophysical Biochem. Res. Comm. 202, 174-80); 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (Turkoglu et al., 1991 Int. J. Biochem. 23, 147-51); octopinen; N-tosyl-L-arginine; and N-p-tosyl-L-arginine methylester. The latter compound is converted in vivo to N-p-tosyl-L-arginine by tissue esterases. "Inhibitors", as used herein, include compounds which themselves inhibit arginase and compounds which in vivo convert to compounds that inhibit arginase. The preferred inhibitors are irreversible.

The inhibitor of arginase preferably is incorporated in a topical composition which includes a non-toxic dermatologically acceptable vehicle or carrier which is adapted to be spread upon the skin.

5 Examples of suitable vehicles are acetone, alcohols, or a cream, lotion, or gel which can effectively deliver the active compound. One such vehicle is disclosed in co-pending application PCT/US93/0506A. In addition, a penetration enhancer may be added to the vehicle to  
10 further enhance the effectiveness of the formulation.

The concentration of the inhibitor of arginase in the composition may be varied over a wide range up to a saturated solution, preferably from 0.1% to 30% by weight or even more; the reduction of hair  
15 growth generally increases as the amount of the inhibitor applied increases per unit area of skin. The maximum amount effectively applied is limited only by the rate at which the inhibitor penetrates the skin. Generally, the effective amounts range from 100 to 3000  
20 micrograms or more per square centimeter of skin.

The composition should be topically applied to a selected area of the body from which it is desired to reduce hair growth. For example, the composition can be applied to the face, particularly to the beard  
25 area of the face, i.e., the cheek, neck, upper lip, and chin. The composition can also be applied to the legs, arms, torso or armpits. The composition is particularly suitable for reducing the growth of unwanted hair in women suffering from hirsutism or  
30 other conditions. In humans, the composition should be applied once or twice a day, or even more frequently, for at least three months to achieve a perceived reduction in hair growth. Reduction in hair growth is demonstrated when the frequency of hair removal  
35 (shaving, tweezing depilatory use, waxing) is reduced, or the subject perceives less hair on the treated site, or quantitatively, when the weight of hair removed by

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shaving (i.e., hair mass) is reduced. Benefits of reduced hair removal frequency include convenience and less skin irritation.

Male intact Golden Syrian hamsters are considered acceptable models for human beard hair growth in that they display oval shaped flank organs, one of each side, each about 8 mm. in major diameter, which growth thick black and coarse hair similar to human beard hair. These organs produce hair in response to androgens in the hamster. To evaluate the effectiveness of a particular arginase inhibitor the flank organs of each of a group of hamsters are depilated by applying a thioglycolate based chemical depilatory (Surgex). To one organ of each animal 10  $\mu$ l. of vehicle alone once a day is applied, while to the other organ of each animal an equal amount of vehicle containing an arginase inhibitor is applied. After thirteen applications (one application per day for five days a week), the flank organs are shaved and the amount of recovered hair (hair mass) from each is weighed. Percent-reduction of hair growth is calculated by subtracting the hair mass (mg) value of the test compound treated side from the hair mass value of the vehicle treated side; the delta value obtained is then divided by the hair mass value of the vehicle treated side, and the resultant number is multiplied by 100.

The above-described assay will be referred to herein as the "Golden Syrian hamster" assay. Preferred compositions provide a reduction in hair growth of at least about 30%, more preferably at least about 45%, and most preferably at least about 60% when tested in the Golden Syrian hamster assay. A number of compositions were tested in the Golden Syrian hamster assay; the results are provided in Table I.

**Table I: Reduction of Hair Growth by Topical Application of Inhibitors of Arginase**

[illegible]

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In vitro studies on inhibition of hair follicle arginase confirmed the biochemical action of the hair growth inhibitory compounds which were selected as inhibitors of hair follicle arginase.

5     Extracts of hair follicles were obtained by excising and sonicating hair follicles obtained from hamster flank organs. The hair follicles from the hamster flank organ were excised and sonicated in a 0.01 M Tris-buffered solution, pH 7.5. The sonicated extracts  
10     were centrifuged at 12,000 x g, and the supernatant was used to measure arginase activity. Final reaction volumes were achieved by using 90 $\mu$ l of the follicle supernatant, which was mixed with 100 $\mu$ l of the Tris buffer (with or without the inhibitor), and 10 $\mu$ l of  
15     20mM arginine incubated for 5 minutes at 37°C. The enzyme reaction was stopped by separating the enzyme from the substrate with a 10,000 molecular weight cut off filter. The product formed, ornithine, was separated from the substrate arginine using reverse  
20     phase HPLC methodology. An aliquot of the reaction was used to generate 20 $\mu$ l of the filtrate which was reacted with 2 $\mu$ l of O-phthalaldehyde (OPA) reagent. This reagent rapidly reacts with all primary amino acids to form fluorescent adducts that are separated on a C18  
25     column (15cm x 3.9mm). The 40 $\mu$ l of derivatized sample is injected into the HPLC column and eluted with a stepwise gradient starting at 100% Buffer A (.024M sodium acetate, .024M sodium phosphate, pH  
30     4.0/methanol/tetrahydrofuran in a ratio of 81:15:4) and ending in a 50/50 mixture of Buffer A and Buffer B (methanol/water/tetrahydrofuran in a ratio of  
35     75:15:10). A fluorescent detector was used to monitor the amino acid peaks as they eluted off the column. Using this method arginine eluted off the column at 20 minutes and ornithine formation when included at a final concentration of 10uM. The magnitude of hair mass inhibition correlated well with in vitro results.



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N-tosyl-L-arginine methylester did not demonstrate in vitro activity but in vitro it is converted to N-tosyl-L-arginine by tissue esterases. The results are provided in Table II.

5                   TABLE II: In Vitro Assay for Inhibitors  
                    of Hair Follicle Arginase

10	Compound (10 $\mu$ M)	% Inhibition
	$\alpha$ -Aminoisobutyric acid	27%
	1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide	64%
	N-Tosyl-L-arginine	64%
	N-p-Tosyl-L-arginine methyl ester	0%
	Octopine	50%
	N <sup>G</sup> -Hydroxy-L-arginine	98%

15                   It will be appreciated by those skilled in the art that the invention can be performed within a wide range of equivalent parameters of composition and conditions without departing from the spirit or scope of the invention or of any embodiment thereof.

C L A I M S

1. A method of reducing mammalian hair growth, comprising  
selecting an area of skin from which reduced hair growth is desired; and  
applying to said area of skin a dermatologically acceptable composition including an inhibitor of arginase in an amount effective to reduce hair growth.
2. The method of claim 1, wherein said inhibitor of arginase comprises alpha aminoisobutyric acid.
3. The method of claim 1, wherein said inhibitor of arginase is N<sup>G</sup>-hydroxy-L-arginine.
4. The method of claim 1, wherein said inhibitor of arginase comprises 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide.
5. The method of claim 1, wherein said inhibitor of arginase comprises octopine.
6. The method of claim 1, wherein said inhibitor of arginase comprises N-tosyl-L-arginine.
7. The method of claim 1, wherein said inhibitor of arginase comprises 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide.
8. The method of claim 1, wherein the concentration of said inhibitor of arginase in said composition is between 1% and 30%.
9. The method of claim 1, wherein the composition is applied to the skin in an amount of from 100 to 3000 micrograms of said inhibitor of arginase per square centimeter of skin.
10. The method of claim 1, wherein the composition is applied to the skin on the face of said mammal.
11. The method of claim 1, wherein the composition provides a reduction in hair growth of at least 30% when tested in the Golden Syrian hamster assay.
12. The method of claim 1, wherein the composition provides a reduction in hair growth of at least 45% when tested in the Golden Syrian hamster assay.

13. The method of claim 1, wherein the composition provides a reduction in hair growth of at least 60% when tested in the Golden Syrian hamster assay.
14. The method of claim 1, wherein said mammal is a human.
15. The method of claim 1, wherein said area of skin comprises an area suffering from hirsutism.
16. The method of claim 1, wherein said composition further comprises a non-toxic, dermatologically acceptable carrier or vehicle.
17. The method of claim 1, wherein said inhibitor of arginase is a compound which itself inhibits arginase.
18. The method of claim 1, wherein said inhibitor of arginase is a compound which converts in vivo to a compound which inhibits arginase when applied topically to skin.
19. The use of an inhibitor of arginase for the manufacture of a medicament for inhibiting mammalian hair growth.
20. The use according to claim 19, wherein said inhibitor is as defined in any one of claims 1 to 7.
21. A method of producing a composition for inhibiting mammalian hair growth, which comprises selecting an inhibitor of arginase, and combining said inhibitor, in an amount effective to reduce hair growth, with a non-toxic, dermatologically acceptable vehicle or carrier.
22. A method according to claim 21, wherein said vehicle or carrier is adapted to be spread upon the skin of a mammal.
23. A method according to claim 21 or 22, wherein said inhibitor is as defined in any one of claims 2 to 7.
24. The new use of an inhibitor of arginase for reducing hair growth.
25. A composition when used for inhibiting mammalian hair growth, which includes an inhibitor of arginase in an amount effective to reduce hair growth and a non-toxic, dermatologically acceptable vehicle or carrier.
26. A composition according to claim 25, wherein said inhibitor is as defined in any one of claims 2 to 7.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 96/18788

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K7/06

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FR 2 609 393 A (SEROBIOLOGIQUES LAB SA) 15 July 1988 see claims 1,2	1-26
A	ARCH. DERMATOL. RES., vol. 259, no. 2, 1977, pages 151-156, XP000645490 H. PRATZEL: "biochemistry of free amino acids in the stratum corneum of human epidermis. I. The arginase reaction" see the whole document	1-26

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

21 March 1997

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/ 18788

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
  
See next page
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

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2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
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Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 96/ 18788

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

In view of the large number of structurally different compounds, which have the property to inhibit arginase, the search had to be restricted for economic reasons. The search was limited to the compounds for which data was given and/or the compounds mentioned in the claims and description and to the general idea underlying the application. (see Guidelines part B, chapter III, paragraph 3.6)

Claim 7 is superfluous because it is identical to claim 4.

### Information on patent family members

PLT/US 96/18788

Form PCT/ISA/210 (patent family annex) (July 1992)